Our Ref.: 408.014-CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:	Examiner: D. Johannsen
MAGDALENA et al	:	
Serial No.:	:	Group: 1634
Filed: Concurrently Herewith	:	
For: FRAGMENTS...COMPLEX	600 Third Avenue	
	New York, NY 10016	
	February 28, 2002	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

Page 1, before line 1, insert

--**PRIOR APPLICATIONS**

This application is a continuation of U.S. Patent Application Serial No. 09/242,588 filed May 20, 1999 which is a 371 of PCT/FR97/01483 filed August 12, 1997.--

Page 10, after line 13, insert the following new paragraph:

--The vectors I-1765 and I-1766 have both been deposited on August 7, 1996 at the CNCM (Collection Nationale de Cultures de Microorganismes, Institute Pasteur, 25 Rue du Docteur Roux, F-75724 Paris, Cedex 15, France (under the Budapest Treaty).--

IN THE CLAIMS:

Cancel claims 1 and add the following claims.

--**28.** A fragment of a nucleic acid specific to mycobacteria of M. tuberculosis complex having a nucleotide sequence selected from

the group consisting of SEQ ID. No: 1, SEQ ID NO. 2 and their complementary sequences.

29. A fragment of a nucleic acid specific to mycobacteria of M. tuberculosis complex having a nucleotide sequence selected from the group consisting of SEQ ID No: 1 and its complementary sequence.

30. A fragment of a nucleic acid specific to mycobacteria of M. tuberculosis complex which fragment is different from BCG, and has a nucleotide sequence selected from the group consisting of SEQ ID No: 2 and its complementary sequence.

31. A cloning or expression vector containing a nucleic acid sequence of claim 28.

32. A vector of claim 31 which is a plasmid selected from the group consisting of pRegX3Bcl and pRegX3Mtl deposited at CNCM under Nos. I-1765 and I-1766, respectively.

33. A nucleotide probe or nucleotide primer that hybridizes under high stringency conditions with one of the sequences of claim 28, its corresponding RNA sequences or its corresponding gene, and that contains a maximum of 21 base pairs.

34. A nucleotide probe of claim 33 comprising 24 consecutive nucleotides selected from the sequences of claim 28.

35. A nucleotide probe of claim 33 comprising sequence SEQ ID No: 1 or its complementary strand.

36. A nucleotide probe of claim 33 comprising two successive sequences SEQ ID NO: 1 followed by a sequence SEQ ID No: 2.

37. A nucleotide probe for detection of specific sequences of

nucleic acids of M. tuberculosis complex other than BCG comprising a sequence of the region of sequence SEQ ID No: 2 comprising the GAG codon in positions 40 to 42 or its complementary strand.

38. A nucleotide probe of claim 37 comprising a sequence composed of 9 base paris upstream and 9 base paris downstream of the GAG codon in positions 40 to 42 or its complementary strand.

39. A nucleotide probe of claim 37 comprising a sequence composed of 9 base paris upstream and 9 base paris downstream of the GAG codon in positions 40 to 42.

40. A nucleotide probe of claim 37 comprising the sequence SEQ. ID No: 2 or its complementary strand.

41. A nucleotide probe of claim 33 labelled by dioxygenin.

42. A nucleotide primer pair for amplification of a specific nucleotide sequence of mycobacteria of M. tuberculosis complex, said pair comprising the nucleotide sequence of sequences adjacent to the senX3-regX3 region in the 3′ of seX3 and 5′ of regX3 regions.

43. A nucleotide primer of claim 42 comprising 19 nucleotides.

44. A nucleotide primer of claim 42 comprising the pair of primers 5′GCGCGAGAGCCCGAACTGC3′ AND 5′GCGCAGCAGAAACGTCAGC3′.

45. In an enzymatic amplification method, the improvement comprising using as the diagnostic probe or primer a fragment of claim 28.

46. In the detection or diagnosis of a strain belonging to M. tuberculosis complex, the improvement comprising as the <u>in vitro</u>

tool, a nucleotide probe or nucleotide primer of claim 33.

47. A method of detecting a mycobacteria stain of M. tuberculosis complex in a biological sample comprising (1) contacting the biological sample to a pair of primers of claim 42 under conditions to effect hybridization of the primers to the specific nucleic acids of mycobacteria strains of M. tuberculosis complex, (2) effecting amplification of the said nucleic acids, (3) contacting the biological sample with a nucleotide probe of claim 33 under conditions for formation of hybridization complexes between the said probe and amplified sequences of nucleic acids and (4) detecting if any hybridization complexes are present, which complexes indicate the presence of a mycobacteria strain of M. tuberculosis.

48. The method of claim 47 wherein the nucleotide probe is that of claim 35.

49. The method of claim 47 wherein the nucleotide probe is that of claim 37.

50. The method of claim 49 effected upon immunodeficient humans to differentiate an infection by BCG from an infection by a virulent mycobacterium of M. tuberculosis complex.

51. The method of claim 50 wherein the human is infected with HIV.

52. A method of identifying groups of mycobacteria belonging to a M. tuberculosis complex comprising (1) contacting the DNA of previously extracted strains of the M. tuberculosis complex with a pair of primers of claims 35 and 42 under conditions permitting a

specific hybridization of the primers with one of the sequences of claim 28 to obtain amplification products and (2) measuring the length of the amplification products obtained.

53. The method of claim 52 wherein the pair of primers are 5'GCGCGAGAGCCCGAACTGC3' AND 5'GCGCAGCAGAAACGTCAGC3'.

54. A kit for <u>in</u> <u>vitro</u> identification of strains of mycobacterial of the M. tuberculosis complex in a biological sample comprising (1) a primer pair for amplification of a specific nucleotide sequence of mycobacteria of M. tuberculosis complex, said pair comprising the nucleotide sequence of sequences adjacent to the senX3-regX3 region in the 3' of seX3 and 5' of regX3 regions.

55. A method of detection and of differential diagnosis of BCG and the members of M. tuberculosis complex in a biological complex comprising:

(1) contacting the biological sample to a nucleotide primer pair for amplification of a specific nucleotide sequence of mycobacteria of M. tuberculosis complex, said pair comprising the nucleotide sequence of sequences adjacent to the senX3-regXe region in the 3' of seX3 and 5' or regX3 regions under conditions to effect hybridization of the primers to the specific nucleic acids of mycobacteria strains of M. tuberculosis complex;

(2) effecting amplification of the said nucleic acids;

(3) contacting the biological sample with a nucleotide probe of two successive sequences SEQ ID No: 1 followed by a sequence SEQ ID NO: 2 under conditions for formation of hybridization complexes

between the said probe and amplified sequences of nucleic acids; and

(4) detecting any first hybridization complexes present; and

(5) determining if said first hybridization complexes are also capable of forming second hybridization complexes with a nucleotide probe for detection of specific sequences of nucleic acids of M. tuberculosis complex other than BCG comprising a sequence of the region of sequence SEQ ID No: 2 comprising the GAG codon in positions 40 to 42 or its complementary strand, the presence of said second hybridization complexes being indicative of the presence of a M. tuberculosis strain different from BCG.

REMARKS

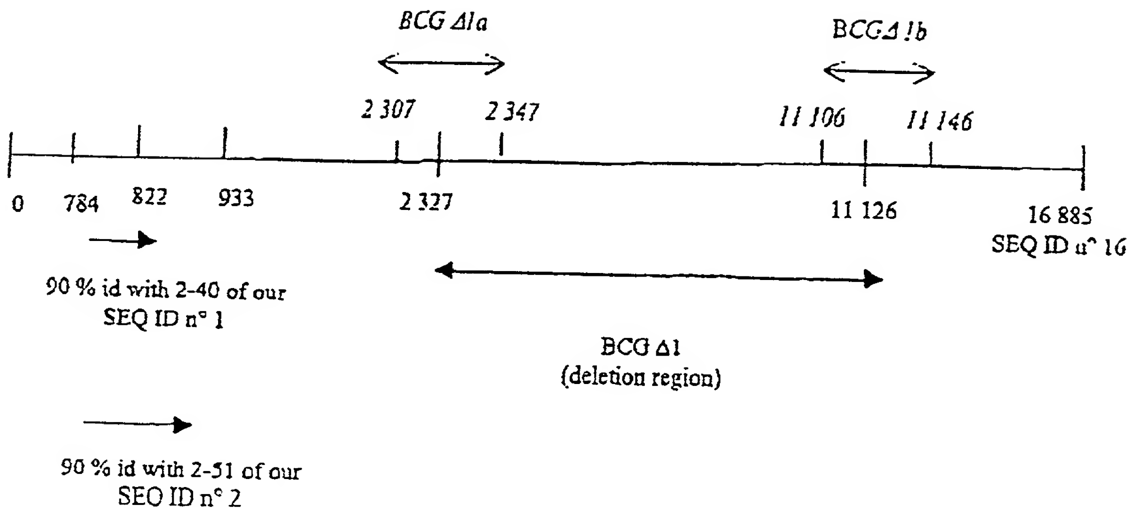
The amendment is presented to insert reference to the parent applications, amend the specification as in the parent application and to rewrite all the claims to avoid the objections to the claims as last filed therein.

In the parent application, Claims 28 to 31, 33, 34, 37 to 39, 41 to 43, 45 to 47, 49, 50 and 53 were rejected under 35 USC 102 as being anticipated by the Stover et al patent. Claims 51, 52 and 55 were rejected under 35 USC 103 as being obvious over the Stover et al patent taken in view of the Van Embden et al reference. Claims 23, 24 and 27 remain rejected over Stover et al in view of Van Embden et al. The Examiner states that the Stover et al patent teaches fragments of

nucleic acids that are capable of hybridizing to SEQ ID No: 1 and No: 2 and the compliments of the said sequence ID numbers under conditions of high stringency as defined in the specification. Many of the Stover et al sequences would reasonably be expected to meet the limitations of the present claims according to the Examiner.

Applicants respectfully traverse these grounds of rejection since Stover et al, taken alone or in view of the secondary art, does not anticipate or render obvious Applicants' invention which relates to the nucleic SEQ ID No: 1 and No: 2 which permits differentiation of BCG from other bacteria of the M. tuberculosis complex. With respect to the Examiner's allegation that Stover et al discloses deleted regions in the BCG genome and that these deleted regions are indicative of an avirulent phenotype, this objection is now moot since amended claims 28 to 30 no longer call for the hybridizing sequences. Moreover, the sequences or probes that can be derived from the SEQ ID No: 16 of Stover et al do not anticipate the probes or primers of the present application.

The Examiner's analysis of the Stover et al patent is apparently effected by several misunderstandings. The teaching of Stover et al can be summarized by the following scheme:



According to Stover et al, the markers or probes that can be derived from the SEQ ID No: 16 of Stover et al may be a) the full length BCGΔ1a or BCGΔ1b or a sequence within these regions as taught in line 67 of column 1 and lines 1 and 3 of column 2 or a sequence selected from an open reading frame (ORF) of the BCGΔ1 deletion sequence (see lines 9 and 10 of column 2), the BCGΔ1 ORFs are depicted in Figure 4.

The nucleotide sequences 784-933 of SEQ ID No: 16 which shows 90% identity with nucleotides 2 to 40 of SEQ ID No: 1 of the present application neither belongs to BCGΔ1a or BCGΔ1b nor to BCGΔ1 and thus, cannot be a marker sequence according to Stover et al. Moreover, if one refers to the general characterization of the sequences according to Stover et al, it is specified that the sequences are either deletion junction sequences or deletion sequences or subsequences within these sequences (lines 50 to 53 of

column 1). A definition of "deletion junction" is recited beginning at line 62 of column 4, it is mentioned that a sequence spans to nucleotides that are immediately adjacent to the deletion sequence. In no event can the Examiner maintain that the nucleotides lined between position 784 to 933 are immediately adjacent to nucleotide 2327 of SEQ ID No: 16.

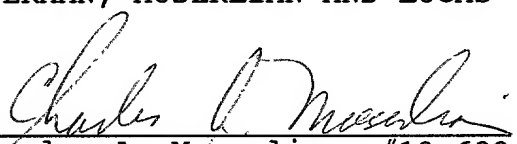
Moreover, Applicants wish to stress that the specification states that "A probe will be selected that hybridizes to the target junction sequences (i.e. BCGΔ1a and BCGΔ1b) or deletion sequences [i.e. BCGΔ1] but not to other microbacterial nucleic sequences under stringent conditions" in lines 37 to 40 of column 12. Within the context of Stover et al, the nucleic acid fragment 784-933 is another microbacterial nucleic acid since this fragment shows no homology to BCGΔ1a, BCGΔ1b or BCGΔ. A probe according to Stover et al is preferably 17 to 25 base in length (see line 59 of column 12). Therefore, a probe comprising the fragment 784-933 would be at least 1,543 bases long, i.e. ranging from nucleotide 784-2327 which is a 100 fold increase as compared to the mentioned length. Therefore, Stover et al does not anticipate or render obvious Applicants' invention.

Stover et al contains no incentive to try and detect a deleted region different from BCGΔ1, BCGΔ12 or BCGΔ3. Therefore, Applicants' invention has an inventive step over Stover et al. The obviousness rejection is deemed to be moot since Van Embden et al

does not teach that a BCG strain can be detected from other M. tuberculosis complex strains by detecting a deletion of nucleotides 40 to 60 in the intercistronic region repeated SEQ ID NO: 1. Therefore, one skilled in the art would not have been led to Applicants' invention by combining the two documents. Therefore, withdrawal of these grounds of rejection is requested.

In view of the proposed amendments to the claims and the above remarks, it is believed that the claims clearly point out Applicants' patentable contribution and favorable consideration of the application is requested.

Respectfully submitted,
BIERMAN, MUSERLIAN AND LUCAS



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CAM:sd
Enclosure: Return Receipt Postcard
Marked-Up Version of Specification

408.014-CON
MARKED-UP VERSION OF SPECIFICATION

Fragments of nucleic acids specific to mycobacteria which are members of the M. tuberculosis complex and their applications for the detection and the differential diagnosis of members of the M. tuberculosis complex

--This is a continuation of U.S. Patent Application Serial No. 09/242,588 filed May 20, 1999 which is a 371 of PCT/FR97/01483 filed August 12, 1997.--

The present invention relates to sequences of nucleic acids of mycobacteria belonging to the *M. tuberculosis* complex.

The invention likewise relates to a method of in vitro detection of strains of mycobacteria belonging to the *M. tuberculosis* complex as well as to a method for a differential diagnosis of strains of the *M. tuberculosis* complex, especially for differentiating the presence of the BCG from that of other members of the complex in a sample.

Approximately, 1.7 billion people or 1/3 of the world population are infected with *M. tuberculosis* (Sudre et al., 1992). In 1990, the estimated number of cases of tuberculosis was 8 million, including 2.9 million deaths (Sudre et al., 1992). These last few years, the number of cases of tuberculosis in the United States and in Europe has increased by 3 to 6% per annum, principally in populations at high risk such as patients suffering from AIDS, chronic alcoholics, the homeless and drug addicts (Barnes et al., 1991).

Taking account of the difficulties in fighting infections by mycobacteria, there is an urgent need to be able to have a specific and sensitive rapid method allowing these infections to be diagnosed. Also, the early detection of *M. tuberculosis* in clinical samples is taking on growing importance in the control of tuberculosis both for the clinical treatment of infected patients and for the identification of exposed individuals at risk.

Detection by PCR (Polymerase Chain Reaction) of specific DNA of species of mycobacteria is probably one of the most promising novel approaches for rapid, specific and sensitive diagnosis (Saiki et al., 1985;